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Lipopolysaccharide-induced liver apoptosis is increased in interleukin-10 knockout mice

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Abstract

Although IL-10 down-regulates pro-inflammatory cytokine secretion by hepatic Kupffer cells, the mechanisms underlying its hepatoprotective effects are not fully clear. This study tested the hypothesis that IL-10 protects the liver against pro-inflammatory cytokines by counteracting their pro-apoptotic effects. Wild type and IL-10 knockout mice were treated with bacterial lipopolysaccharide and sacrificed 1, 4, 8, and 12 h later. Plasma ALT activity was measured as a marker of liver injury. Liver pathology and TUNEL response were assessed by histology. Plasma levels and whole liver mRNA levels were measured for TNF-α, IL-1β, TGF-β1, IL-10, and their respective receptors. Hepatic mRNA levels were measured for several pro-apoptotic adaptors/regulators, including FasL, Fas receptor, FADD, TRADD, Bad, Bak, Bax, and Bcl-X_S, and anti-apoptotic regulators, including Bcl-w, Bcl-X_L, Bcl-2, and Bfl-1. Caspase-3 activity in the liver was determined as well as immunohistochemistry for IL-1RII, TGF-βRII and Fas receptor. At all time points the livers from IL-10 knockout mice displayed a significantly increased number of apoptotic nuclei compared to wild type mice. Changes in plasma cytokine levels and their liver mRNA levels were consistent with suppression by IL-10 of pro-inflammatory cytokine secretion. In addition, pro-inflammatory cytokine receptor mRNA levels (TNF-α, TGF-β, and IL-1β) were markedly up-regulated by LPS at all time points in IL-10 knockout mice as compared to wild type mice. Expression of the pro-inflammatory cytokine receptor IL-1RII was similarly increased as shown by immunostaining. The mRNA levels of a typical pro-apoptotic cytokine, TRAIL, were increased and LPS also up-regulated the mRNA expression of other apoptotic factors to a larger extent in IL-10 knockout mice than in their wild type counterparts, suggestive of an IL-10 anti-apoptotic effect. In the livers of knockout mice, markedly increased caspase-3 activity was already evident at the 1-h time point following LPS administration, while in the wild type animals this increase was delayed. Immunostaining also indicated that LPS increased hepatic expression of the pro-apoptotic receptors Fas and TGF-βRII in IL-10 knockout mice. The data presented in this study show that: (i) IL-10 modulates not only the secretion of pro-inflammatory cytokines, but also the receptors of these cytokines, and ii) IL-10 protects the liver against LPS-induced injury at least in part by counteracting pro-inflammatory cytokine-induced liver apoptosis.

Keywords: Interleukin-10; Liver; Apoptosis; Lipopolysaccharide; Kupffer cell

1. Introduction

It has become increasingly evident that potent activating stimuli, such as Gram-negative bacterial lipopolysaccharide (LPS), induce simultaneous secretion, frequently by the same cells, e.g., macrophages in different tissues, of two functionally heterogeneous groups of cytokines: pro-inflammatory, e.g., TNF-α, IL-1-α, IL-1β, IL-6, TGF-β and anti-inflammatory, e.g., IL-10 [1,2]. Under experimental and clinical conditions, the balance seems to be shifted toward a predominant inflammatory response as a result of the action of pro-inflammatory cytokines. The biological significance of the simultaneous secretion of anti-inflammatory cytokines is less well understood.

Recombinant human IL-10 has been produced and is currently being tested in clinical trials. This includes rheumatoid arthritis, inflammatory bowel disease (Crohn's disease), psoriasis, organ transplantation and chronic hepatitis C [3,4]. IL-10 is a pleiotropic cytokine which down-regulates the secretion of pro-inflammatory cytokines by cells in general and especially inflammatory cells such as liver resident macrophages or

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Kupffer cells [1,5]. Numerous studies have described the biological effects of IL-10 on the liver (see review by Le Moine et al. [6]). IL-10 protects the liver against a number of injurious agents, such as Gram-negative bacterial LPS and D-galactosamine [7–9], concanavalin A [10,11], cold-ischemia–reperfusion [12,13], acetaminophen [14], and CCl_4 [15]. Although these conditions are associated with increased liver apoptosis, a specific role for IL-10 in protecting the liver from apoptosis has not been investigated. IL-10 protection has been thought to be primarily mediated by down-regulation of pro-inflammatory cytokine secretion [8]. However, pro-inflammatory cytokines may be detrimental to the liver by several mechanisms such as increases in oxidative stress (TNF-α), apoptosis (TNF-α and TGF-β), mitochondrial dysfunction (TNF-α) and others, thereby leading to tissue necrosis.

The objectives of this study were thus: (i) to determine the time course of pro-inflammatory cytokine secretion in IL-10 knockout mice and their wild type counterparts upon stimulation with LPS, and (ii) to establish a link between modulation by IL-10 of pro-inflammatory cytokine secretion and liver apoptosis.

2. Materials and methods

2.1. Animals

The experimental protocols were in compliance with the *Guide for the Care and Use of Laboratory Animals* (National Research Council, National Academy Press, Washington, D.C., 1996), as approved by the *Institutional Animal Care and Use Committee* of the Veterans Affairs Medical Center, Lexington, KY, USA.

Male, C57BL/10 mice, weighing 25 g, were purchased from Harlan Laboratories, Indianapolis, IN. IL-10 knockout C57BL/10 mice were bred in our animal facility and matched in age with wild type counterparts. Gram-negative bacterial LPS (*Escherichia coli*, O26:B6, Sigma Chem Co., St. Louis, MO) was suspended in sterile saline and injected intraperitoneally in a dose of 1.0 $mg \cong kg^{-1}$ body weight (at a concentration of 0.25 $mg \cong mL^{-1}$). One group of mice in each of the wild type and IL-10 knockout series received sterile saline only and were sacrificed 1 h after injection. These mice served as controls to LPS-injected mice. All animals had free access to food and water throughout the entire experimental protocol.

2.2. Blood and liver sampling

One, 4, 8 and 12 h after LPS injection, the animals were anesthetized with Na-pentobarbital (Nembutal^R; 80 $mg \cong kg^{-1}$ body weight), the abdominal cavity opened and blood (0.4–0.6 mL) sampled from the exposed inferior vena cava with the aid of citrate-containing syringes. The liver was flushed with 2–3 mL ice-cold buffered saline (NaCl 100 mM; HEPES, 20 mM, pH 7.4) through the portal vein, resected, and blotted on a filter paper. The small left lobe was placed immediately in 3.5% formalin for histology while the rest of the liver was placed in liquid nitrogen for biochemical assays. Blood was centrifuged at 13,000×*g* for 10 min, at 4 °C, and the plasma stored in small aliquots in Eppendorf tubes for future cytokine assays.

2.3. Biochemical assays

2.3.1. Assay of alanine:2-oxoglutarate aminotransferase (ALT, EC 2.6.1.2)

Immediately after plasma isolation, this enzyme was assayed spectrophotometrically with a kit from Sigma Chem. Co. (St. Louis, MO).

Total RNA extraction from the liver was performed using TRIzol reagent according to the manufacturer's instructions (GIBCO BRL, Life Technologies, Rockville, MD). The final purification step was performed using Qiagen minicolumns (Qiagen, GmbH, Hilden, Germany) according to the manufacturer's instructions.

RNAse protection assay for the determination of mRNA abundance was performed using templates and reagents from BD Pharmingen (San Diego, CA). Twenty μg of total RNA were applied on a lane and gel was run as recommended by the template and reagent provider. The gels were scanned with the aid of a Phosphorimager scanner (Molecular Dynamics, Sunnyvale, CA). Optical density of the bands was quantified using the ImageQuant computer assisted program (Molecular Dynamics, Sunnyvale, CA). The data are expressed as the ratio of a specific mRNA band optical density to the band of a housekeeping gene, L32, a ribosomal protein.

2.3.2. Caspase-3 activity

This was measured with a fluorometric assay using a commercial kit (R&D Systems, Minneapolis, MN). Briefly, liver extracts were prepared by homogenization of approximately 100 mg liver tissue with 1 mL lysis buffer (provided with the kit) followed by sonication (two bursts each of 30 s at 50 Ws) and centrifugation for 30 min at 30,000×*g*. All operations were performed on ice or at 4 °C. The supernatant was used for caspase-3 activity assay. The protein concentration in the reaction mixture was between 0.8 and 1.2 mg. The reaction was linear versus time at least for 90 min ($r^2 = 0.989–0.997$).

2.3.3. Protein assay

This was performed using the Bradford procedure with reagents from BioRad (Hercules, CA) and bovine serum albumin as standard.

2.3.4. Plasma cytokines

These were measured using commercially available kits from R&D Systems, Minneapolis, MN).

2.4. Liver histology and immunohistochemistry

Hematoxylin–eosin staining was used for histologic assessment of the liver. The TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling) assay was used to quantify liver apoptosis and was performed using a kit from Intergen (Purchase, NY) according to the manufacturer's instructions. Apoptotic nuclei were counted in a blinded fashion in 6 low-magnification fields for each liver and for 4 livers in each group by two individuals. For immunohistochemistry slides were deparaffinized in a standard fashion, washed in PBS, and then microwaved in Retrieval Solution (DAKO, Carpinteria, CA) for 5 min, washed in PBS, incubated in 0.3% H_2O_2–methanol solution for 10 min, and then washed again in tap water. Slides were then incubated for 1.5 h at room temperature in a blocking solution of normal serum, then rinsed and incubated with primary antibody at 4 °C overnight. Rabbit polyclonal IgG for FAS and TGFâRII, and rat monoclonal IgG for IL-1RII were obtained from Santa Cruz Biotechnology (Santa Cruz, CA) and Pharmingen (San Diego, CA) separately. The Elite ABC kit (Vector Laboratories, Burlingame, CA) was used with biotinylated secondary antibodies and biotin-conjugated horseradish peroxidase and was developed with a 3–3′-diaminobenzidine solution per the manufacturer's instructions. The slides were then counterstained with Dako Methyl Green (DAKO, Carpinteria, CA).

2.5. Statistics

The results are expressed as mean ± standard error of the mean. For assessment of statistical significance Student's *t* test was calculated and $P \leq 0.05$ was accepted as indicating a significant difference between the compared groups.

3. Results

3.1. Plasma ALT activity and liver histology

Data depicted in Fig. 1 show higher levels of ALT in the plasma of IL-10 knockout mice and a significant difference between the groups was seen at the 8-h time point. There were

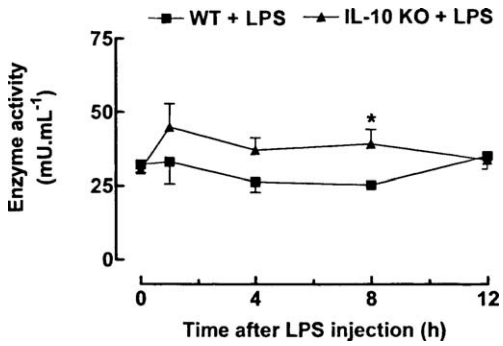

Fig. 1. Effect of LPS treatment on plasma ALT activity in wild type (WT) and IL-10 knockout (KO) mice. Plotted are mean±S.E.M. (vertical bars) for 5–7 animals in each group. *$P<0.05$ versus WT at 8 h. Note that the 0-h time point represents, here and in subsequent graphs, the values obtained in saline-treated animals. Abbreviations in this graph will be used throughout the following figures.

no histologically evident changes induced by LPS administration in either wild type or in IL-10 knockout mice (data not shown).

3.2. Liver TUNEL response

Starting at 4 h after injection, LPS treatment induced a significant increase in the number of apoptotic nuclei in the livers of both wild type and IL-10 knockout mice. The effect was most pronounced in the IL-10 knockout group and reached a peak at 12 h when 11.8±1.9% of total nuclei appeared apoptotic (Fig. 2). Representative micrographs illustrating these effects are presented in Fig. 3. The observation that increased apoptosis was not associated with markedly increased plasma ALT activity can be attributed to the following factors. Plasma ALT activity after intraperitoneal injection of LPS in the dose used in this study (1 mg/kg b.w.) is not expected to increase earlier than 24 h after injection. Even then, the increase is moderate as compared to the increase observed after intravenous injection of same dose of LPS. Furthermore, apoptosis is not associated with spill-over of the cellular content and the

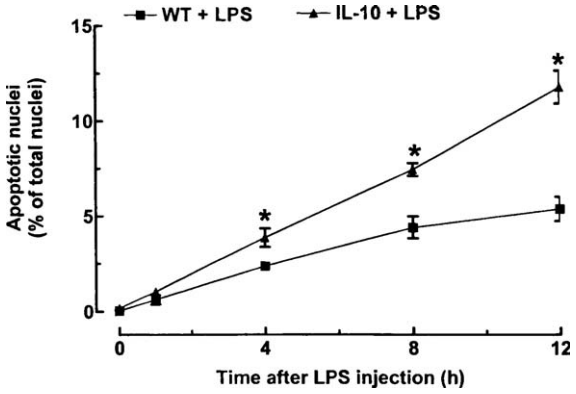

Fig. 2. Effect of LPS treatment on the number of apoptotic nuclei in the liver. Plotted are means±S.E.M. (vertical bars) for 4 animals in each group. *$P<0.05$ versus WT, LPS-treated mice. The difference between 0 h time and other time points was significant ($P<0.05$) for both groups, with the exception of the 1-h time point.

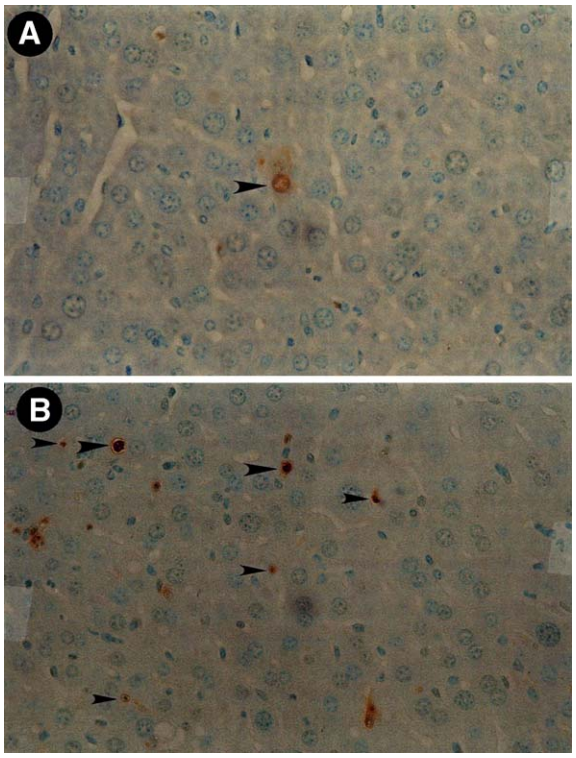

Fig. 3. TUNEL response of the liver in a WT, saline-treated mouse (A), and an IL-10 KO, LPS-treated mouse (B). Animals were sacrificed 12 h after LPS injection. Note the presence in A of one apoptotic nucleus (arrowhead) which still maintains its original size. (panel B) Two types of apoptotic nuclei can be distinguished: large (large arrowheads), approximate in size to non-apoptotic nuclei and small (small arrowheads) which most likely represent apoptotic nuclei in the final stage of phagocytosis. Original magnification: ×400. Numerical data on apoptotic nuclei are presented.

degree of apoptosis may not be high enough to lead to hepatocellular necrosis and would therefore not be associated with increased ALT.

3.3. Plasma cytokine and liver cytokine and cytokine receptor mRNA levels

The plasma levels of two pro-inflammatory cytokines, i.e. TNF-α and IL-1β, and one anti-inflammatory cytokine, IL-10, were measured. TNF-α in plasma increased significantly in the wild type mice 1 h after LPS injection and then decreased toward the basal level at 8 h (Fig. 4). In IL-10 knockout mice, TNF-α levels were significantly increased at all time points as compared to both saline-injected animals (zero time point on the graph) and to wild type animals (Fig. 4). IL-1β displayed different kinetics from that of TNF-α. A significant increase in IL-1β levels was first observed at 8 h; levels then declined to the pre-LPS injection level (zero time point) in the wild type mice, but continued to increase in IL-10 knockout mice. In the latter group, similar to TNF-α, the IL-1β levels peaked at 8 h and then declined (Fig. 4). TNF-α mRNA kinetics closely resembled the plasma cytokine levels except that the difference between wild type and IL-10 knockout mice was statistically significant at the 4-h time point only (Fig. 4). IL-1β mRNA increases preceded that of the cytokine plasma levels and was significantly

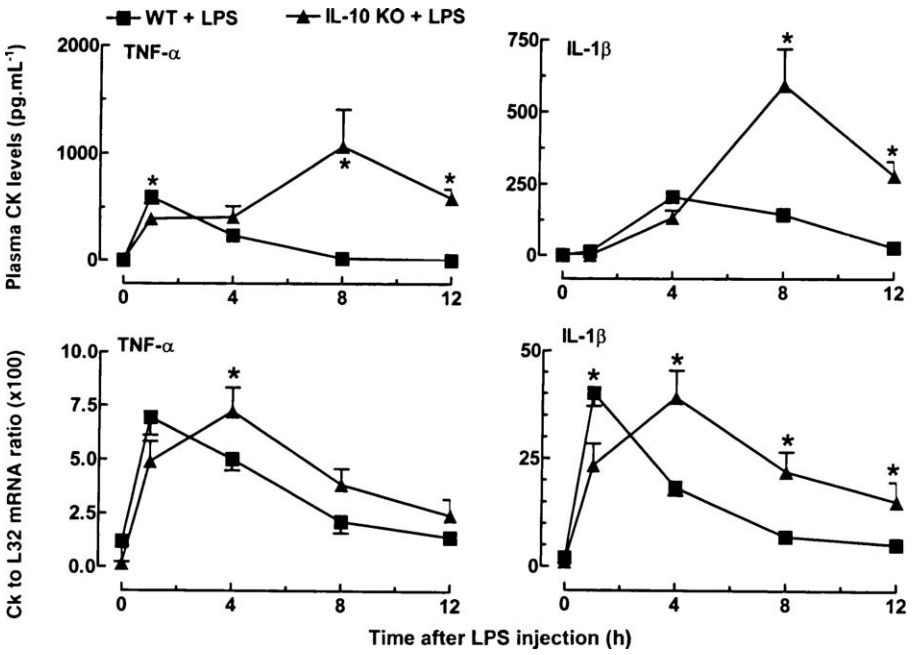

Fig. 4. Plasma TNF-α and IL-1β levels and hepatic mRNA expression in WT, LPS-treated and IL-10 KO, LPS-treated mice. Plotted are means±S.E.M. (vertical bars) for 6 animals in group. *$P<0.05$ versus WT group; CK, cytokine.

increased in IL-10 knockout mice up to 12 h, with the exception of the 1-h time point after LPS injection. The peak of the increase in IL-1β mRNA also occurred earlier in the wild type (1 h) than in the IL-10 knockout mice (4 h) (Fig. 4).

IL-10 levels in plasma were, as expected, detected only in wild type mice and increased to a peak 1 h after LPS treatment followed by a gradual decline without reaching the pre-LPS levels (Fig. 5). IL-10 mRNA kinetics resembled plasma IL-10 protein kinetics very closely (Fig. 5).

The kinetics of hepatic mRNA expression levels of TNF-α and IL-1 receptors are presented in Fig. 6. TNF-α receptor p55 (receptor I) mRNA increased to a plateau 4 h after LPS with small differences between wild type and IL-10 knockout mice. A trend to a return to the basal levels was seen in wild type, but not in IL-10 knockout mice (Fig. 6). TNF-α receptor p75 (receptor II) mRNA increased significantly only in IL-10 knockout animals and maintained a plateau from 4 to 12 h after LPS treatment.

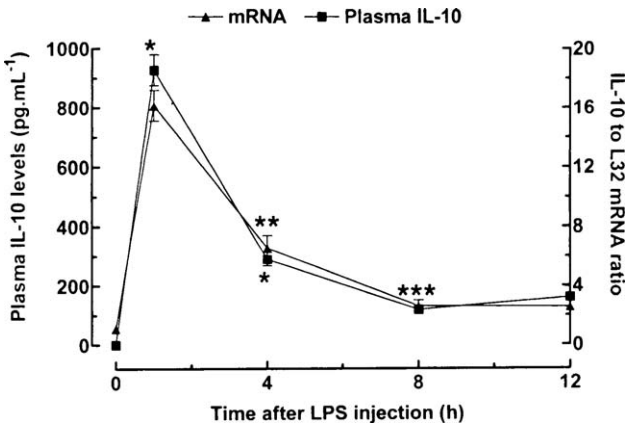

Fig. 5. Plasma and hepatic mRNA levels of IL-10 in WT, LPS-treated mice. Plotted are means±S.E.M. (vertical bars) for 6 animals in group. *$P<0.05$ versus zero time; **$P<0.05$ versus 1 h time point; ***$P<0.05$ versus 4 h time point for both protein and mRNA levels.

IL-1β RI and RII transcripts were increased by LPS treatment in both wild type and IL-10 knockout mice with the effect being much more pronounced in the latter group (Fig. 6). A clear trend to a return to basal levels could again be seen in wild type mice while in IL-10 knockout mice the increase reached a plateau that lasted until the 12-h time point (Fig. 6).

Messenger RNA levels of TRAIL, a typical apoptotic ligand involved in apoptosis following ligation of death receptors, increased to a peak 4 h after LPS in both wild type and IL-10 knockout mice. Levels decreased to the basal level (at 8 h) in the wild type mice but not in IL-10 knockout mice (Fig. 7).

Hepatic transcript levels were also measured in this study for TGF-β1 and its two receptors, RI and RII. This cytokine is a potent apoptotic signal for hepatocytes [17]. While little or no effect of LPS could be detected on TGF-β mRNA in the wild type mice, LPS markedly increased TGF-β mRNA expression in IL-10 knockout mice to a peak at 8 h followed by some decrease towards basal levels (Fig. 8). Similar kinetics were seen for the TGF-βRI and RII transcripts which increased in IL-10 knockout mice to reach a plateau 4 h after LPS injection; however, no trend to recovery was evident (Fig. 8).

3.4. Apoptosis adaptors/regulators mRNA expression

RNAse protection assays were performed to examine the hepatic mRNA expression of apoptotic regulators and adaptors. The selected apoptotic markers include apoptotic ligands (FasL, TRAIL), plasma membrane apoptotic receptors (Fas), intracellular adaptors (FADD and TRADD), and several intracellular

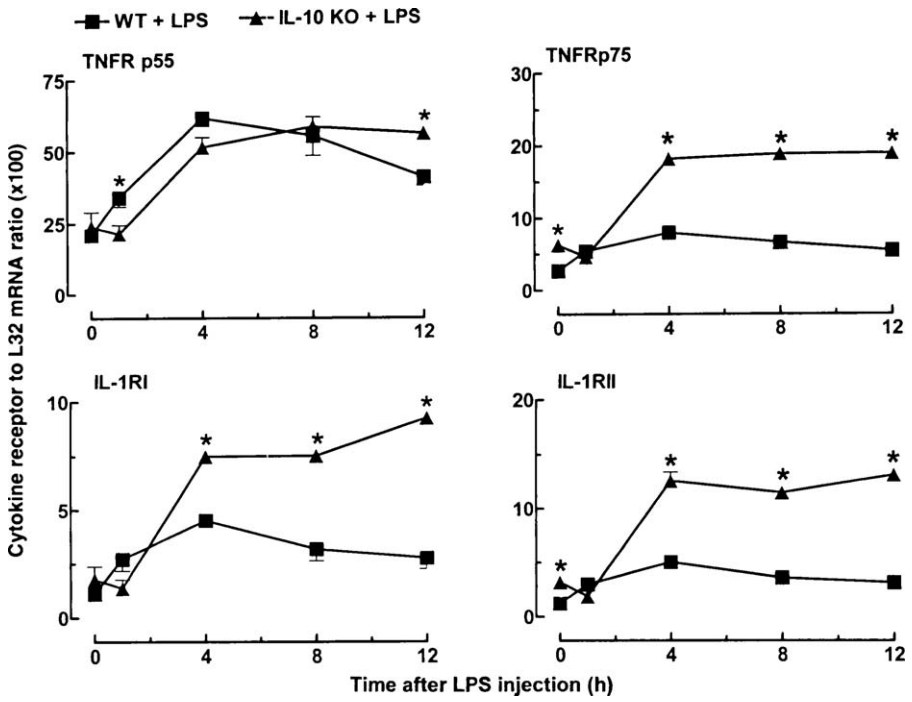

Fig. 6. TNF-α and IL-1β receptor mRNA expression in the livers of WT and IL-10 KO, LPS-treated mice. Plotted are means±S.E.M. (vertical bars) for 4 animals in group. *$P<0.05$ versus WT group.

regulators of apoptosis, including anti-apoptotic (Bcl-w, Bcl-2, Bcl-X_L and Bfl-1) and pro-apoptotic (Bad, Bak, Bax, and Bcl-X_S) regulators.

LPS treatment increased FasL expression in both wild type and IL-10 knockout mice with the effect being more pronounced in the latter. A decrease toward basal levels was seen only in wild type mice (Fig. 9). Similar kinetics were observed for Fas receptor expression (Fig. 9). mRNA levels for two apoptotic adaptors, FADD and TRADD, were up-regulated by LPS in both wild type and IL-10 knockout mice. The increase was more evident in the IL-10 knockout group compared with wild type animals (Fig. 10). LPS treatment similarly markedly increased the expression of anti-apoptotic regulators, such as Bcl-X_L, Bfl-1 and Bcl-2 in IL-10 knockout mice but had little or no effect on Bcl-w mRNA (Fig. 11).

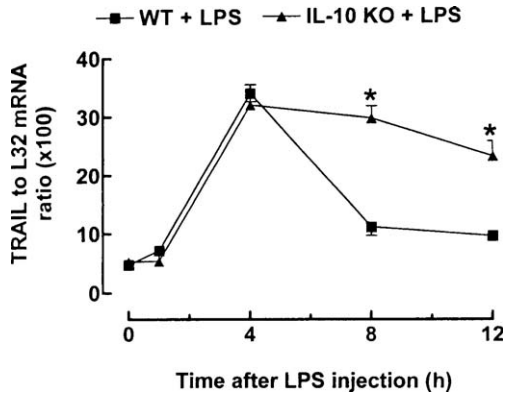

Fig. 7. Effect of LPS treatment on TRAIL mRNA expression in the liver. Plotted are means±S.E.M. (vertical bars) for 4 animals in each group. *$P<0.05$ versus WT group.

Among pro-apoptotic regulators, LPS affected Bak and Bcl-X_S expression most prominently. Levels were markedly up-regulated by LPS in IL-10 knockout mice and to a lesser extent in the wild type mice (Fig. 12). Bax and Bad were also up-regulated by LPS but there was little or no difference evident between the wild type and IL-10 knockout animals (Fig. 12).

3.5. Caspase-3 activity and immunohistochemistry

LPS administration increased caspase-3 activity in both IL-10 knockout and wild type mice (Fig. 13). However, this increase occurred much earlier in knockout mice and was already evident at the first 1 h time point after LPS, while in wild type mice, a significant increase was only seen at 8 h after LPS injection. At all time points, with the exception of 12 h, the caspase-3 activity was significantly higher in IL-10 knockout mice than in the wild type animals (Fig. 13). In agreement with the mRNA data obtained by RNAse protection assays, immunohistochemistry showed LPS markedly increased hepatic expression of the pro-inflammatory cytokine receptor IL-1RII, as well as the pro-apoptotic receptors Fas and TGF-βRII in IL-10 knockout mice 8 h after administration (Fig. 14).

4. Discussion

This study describes how the absence of IL-10, an important anti-inflammatory cytokine, increases the response of the liver to exposure to Gram-negative bacterial LPS in terms of pro-inflammatory cytokine secretion and liver apoptosis. IL-10 knockout mice served as the animal model and plasma and mRNA expression levels for pro-inflammatory cytokines and their receptors were measured. Immunostaining of selected

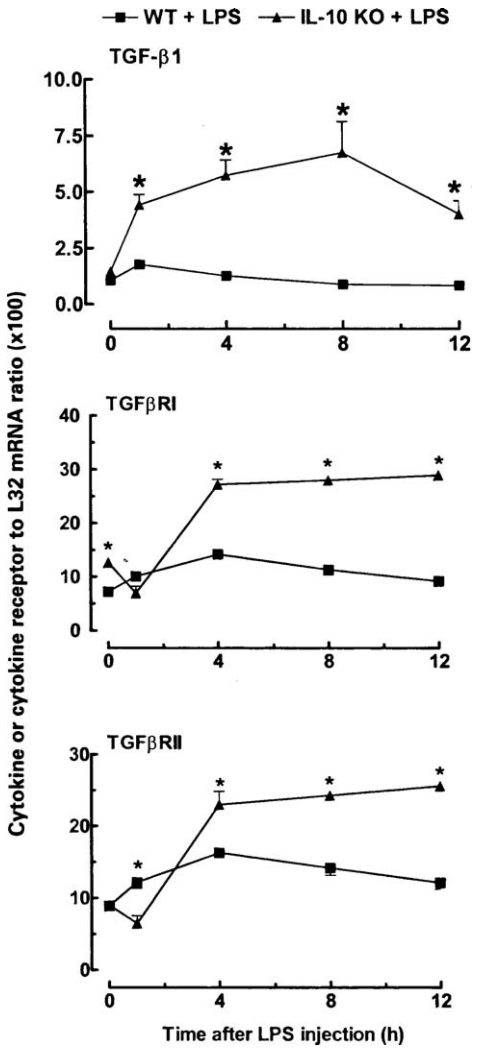

Fig. 8. TGF-β 2 and TGFβ receptor I and II mRNA expression in the livers of mice treated with LPS. Plotted are means ± S.E.M. (vertical bars) for 4 animals in a group. *$P<0.05$ versus WT group.

relevant receptors was also performed. The pro-inflammatory cytokines TNF-α and TGF-β are potent apoptotic signals for liver cells, and their secretion is modulated by IL-10, [16–18]. The apoptotic response of the liver was therefore determined in this experimental context.

Several studies document the down-regulation of pro-inflammatory cytokine secretion by IL-10. The secretion of pro-inflammatory cytokines, measured as either plasma protein concentration and mRNA level (TNF-α and IL-1β), or as mRNA level only (TGF-β1) follows a general pattern consistent with regulation by IL-10 of their respective rates of synthesis and/or secretion. Higher LPS-induced cytokine protein and mRNA levels followed by a marked delay in the return to basal levels are present in IL-10 knockout mice as compared with their wild type controls. In addition, a significant increase in plasma and hepatic mRNA IL-10 levels occur either earlier or simultaneously with those changes seen in plasma or liver mRNA levels of pro-inflammatory cytokines. This suggests that much of the kinetics of the latter may be regulated by IL-10. Our data agree with studies that

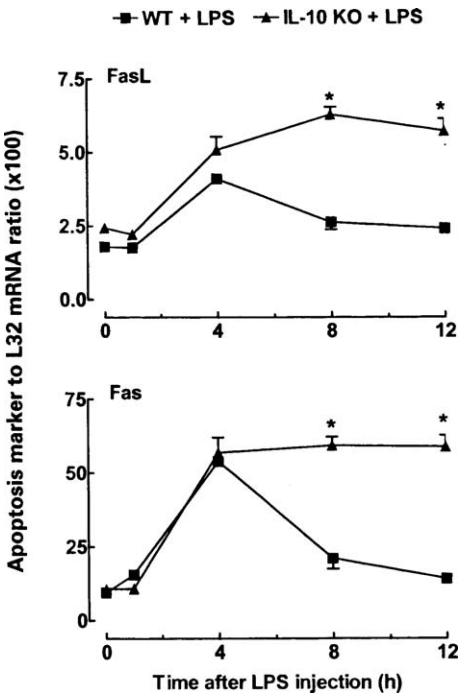

Fig. 9. Effect of LPS treatment on hepatic mRNA expression of FasL and Fas receptor in WT and IL-10 KO mice. Plotted are means ± S.E.M. (vertical bars) for 4 animals in a group. *$P<0.05$ versus WT group.

show that IL-10 suppresses the secretion of TNF-α by Kupffer cells [1]. These cells are probably the most important hepatic target for IL-10 regulation of pro-inflammatory cytokine

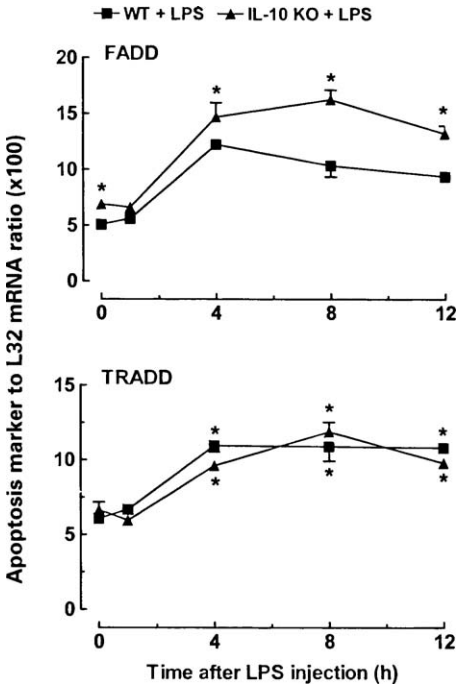

Fig. 10. Effect of LPS treatment on hepatic mRNA expression of FADD and TRADD in WT and IL-10 KO mice. Plotted are means ± S.E.M. (vertical bars) for 4 animals in each group. For FADD: *$P<0.05$ versus WT group; for TRADD: $P<0.05$ versus 0 time point. No statistically significant differences were observed between the groups.

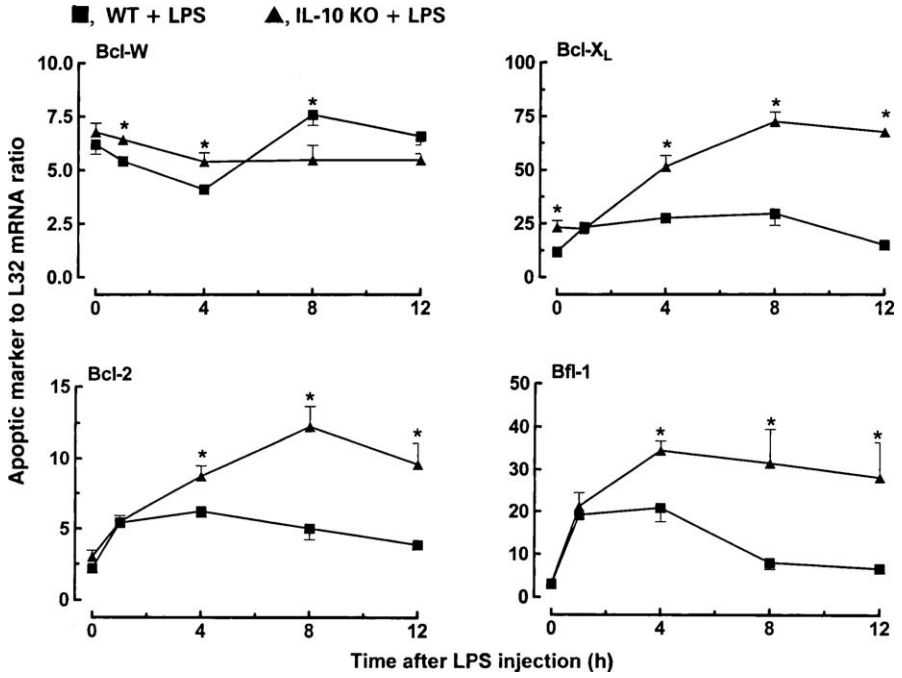

Fig. 11. Effect of LPS treatment on hepatic mRNA expression of anti-apoptotic regulators in WT and IL-10 KO mice. Plotted are means±S.E.M. (vertical bars) for 4 animals in a group. *$P<0.05$ between the groups.

secretion. However, other liver cell types, such as the hepatocyte [19,20], sinusoidal endothelial cell [21–23], and stellate cell [24] also secrete pro-inflammatory cytokines and their synthesis/secretion may be regulated by IL-10.

An intriguing aspect is the kinetic discrepancy between plasma levels of TNF-α and IL-1β and their respective mRNA expression levels in the liver. The kinetics was similar up to 4 h after LPS administration, after which the mRNA levels declined while plasma levels of the cytokines continued to increase. Such a kinetic divergence may be explained by differences in mRNA and protein stability and half-lives as affected by IL-10 regulation. In addition mRNA expression of

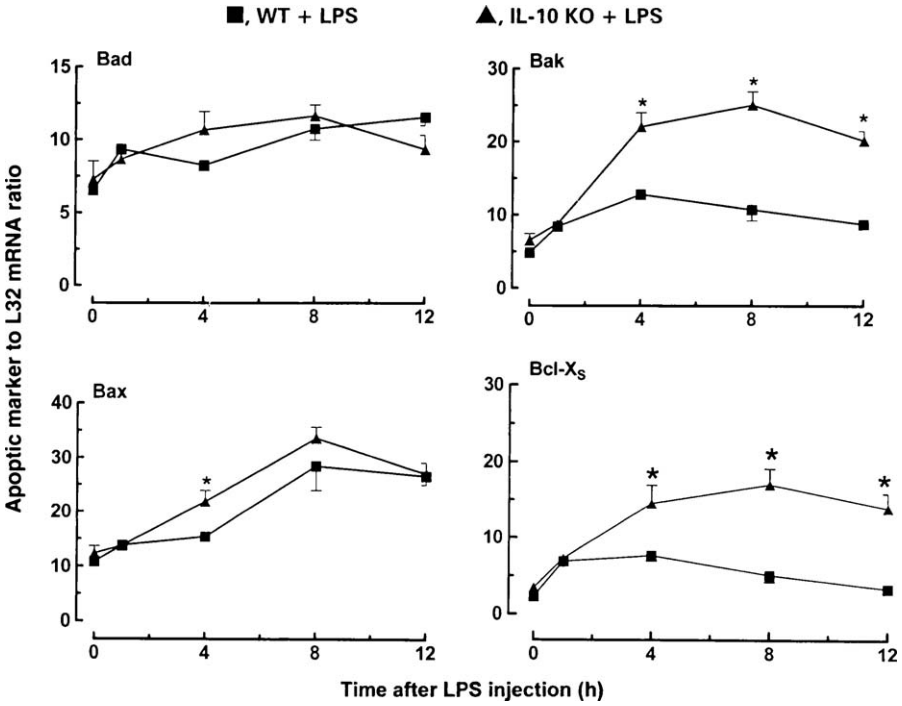

Fig. 12. Effect of LPS treatment on hepatic mRNA expression of pro-apoptotic regulators in WT and IL-10 KO mice. Plotted are means±S.E.M. (vertical bars) for 4 animals in each group. *$P<0.05$ between the groups.

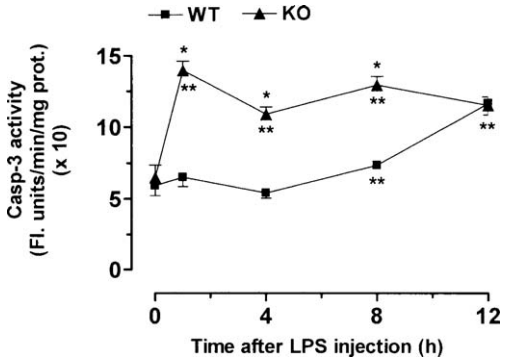

Fig. 13. Effects of LPS on caspase-3 activity in the liver of WT and IL-10 KO mice. Plotted are means ± S.E.M. (vertical bars) for 5–6 animals in each group and time point. *$P<0.05$ between the groups; **$P<0.05$ versus 0 time point. Abbreviations: Casp-, caspase; Fl., fluorescence.

pro-inflammatory cytokine receptors, is also modulated by IL-10. This observation also applied to hepatic protein expression of the IL-1RII molecule as shown by immunostaining. Down-regulation of hepatic TNF-α and IL-1β receptor levels may represent another mechanism whereby IL-10 protects the liver against pro-inflammatory cytokines. To our knowledge, this aspect of IL-10 biological activity has not been thus far described.

LPS-induced pro-inflammatory cytokines have a number of deleterious effects on the liver, including augmentation of apoptosis. This establishes a link between the effects of pro-inflammatory cytokines, IL-10 and liver apoptosis. IL-10 may protect the liver directly by down-regulating apoptotic cytokine secretion and indirectly by counteracting the pro-apoptotic action of pro-inflammatory cytokines.

In this study the augmentation of apoptosis, as judged by the number of apoptotic nuclei, coincided with the level of caspase-3 activity in the liver. Caspase-3 is an effector caspase and is recruited especially when apoptosis is triggered by extrinsic signals, as seems to happen in the case of LPS. In a recent study [25] we showed that LPS induces liver apoptosis in rats via extrinsic signaling, most likely through apoptotic cytokines; as opposed to alcohol which triggers liver apoptosis mainly via intrinsic signals. The similar increasing trends in the number of apoptotic nuclei and the activity of an effector caspase support the contention that the livers of IL-10 knockout mice have an enhanced ability to mount an apoptotic response to appropriate signals. Furthermore, IL-10 may physiologically act as a factor that opposes apoptosis.

This study shows that the processes of pro-inflammatory cytokine kinetics and induction of liver apoptosis parallel each other. It is generally believed that the intracellular apoptotic machinery is readily available to liver cells to respond promptly to intracellular or extracellular apoptotic signals. De novo synthesis of apoptotic proteins may therefore not be necessary for the liver to mount an apoptotic response [26,27]. However, apoptotic signals such as extracellular ligands, e.g., pro-apoptotic cytokines, and their membrane receptors can be up-regulated in the liver by deleterious agents such as LPS. Our findings indicate that pro-apoptotic cytokines such as FasL, TNF-α, TGF-β1 and TRAIL ligand, and their receptors, i.e., Fas, TNFR p75, TGF-βRI and RII, were induced by LPS to a significantly larger extent in IL-10 knockout as compared to wild type control mice. Immunostaining similarly showed increased protein expression of Fas and TGF-βRII. We and others [28,29] have shown previously that Fas receptor and FasL mRNA are up-regulated in the liver by LPS. While these results are not conclusive for the regulation by IL-10 of apoptotic factors, they do indicate a possible link between the biological action of IL-10 and the apoptotic response of the liver to extracellular signals, thereby extending prior knowledge of IL-10 biological activity.

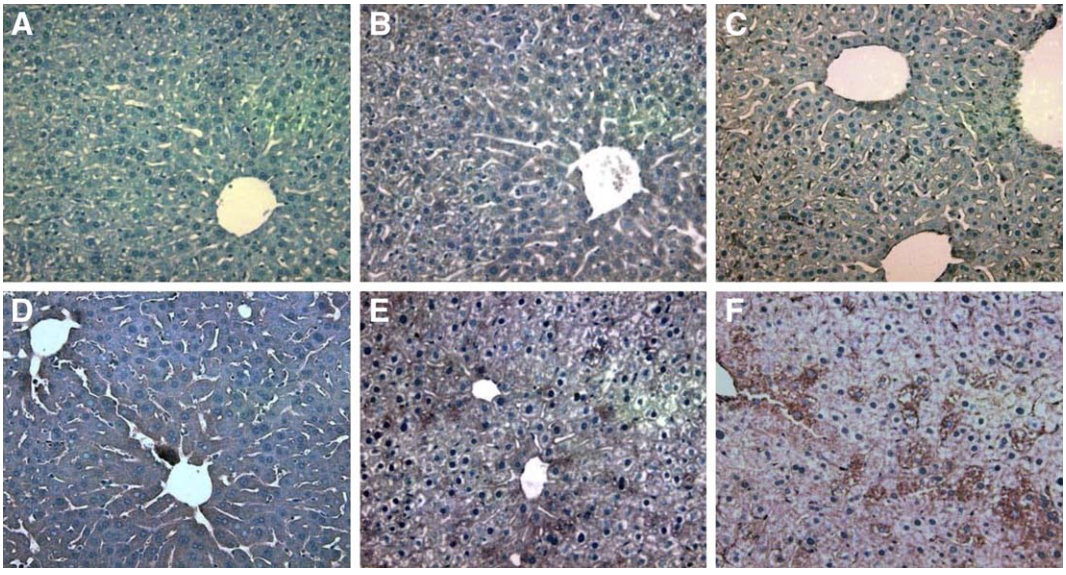

Fig. 14. Liver immunohistochemistry of WT, LPS-treated (A–C), and IL-10 KO, LPS-treated mice (D–F), sacrificed 8 h after saline/LPS injection. A, D IL-1RII; B, E TGF-βRII; C,F Fas receptor. Original magnification: ×200.

Analysis of the expression kinetics of several intracellular apoptotic factors, such as adaptors, e.g., FADD and TRADD, and regulators (both pro- and anti-apoptotic), shows that mRNA levels changed both in response to LPS and the lack of IL-10. In agreement with more extensive apoptosis in IL-10 knockout mice, two pro-apoptotic regulators, Bak and Bcl-X_S, were markedly up-regulated by LPS treatment. It is also interesting to note that up-regulation of anti-apoptotic factors, i.e. Bcl-X_L, Bcl-2 and Bfl-1, was similarly more accentuated in the IL-10 knockout mice than in their counterparts, even though liver apoptosis was more evident in IL-10 knockout mice than in the wild type controls. Up-regulation of antiapoptotic factors/adaptors during enhanced apoptosis has been observed previously (e.g., [26]) and can be interpreted as a tendency of cells to oppose apoptotic death.

In conclusion, our study in IL-10 knockout mice indicates that: (i) pro- and anti-inflammatory cytokines are secreted simultaneously upon stimulation with LPS in vivo; (ii) IL-10, an anti-inflammatory cytokine, markedly inhibits the synthesis and secretion of a number of pro-inflammatory cytokines and down-regulates expression of their receptors; and (iii) IL-10 protects the liver partially by decreasing liver apoptosis. This attenuation occurs as an end result of the down-regulation of both apoptotic cytokines and their receptors, rather than through a direct anti-apoptotic effect on liver cells.

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